Analysis of the immunotherapy model for glioblastoma multiforme brain tumour

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1 Introduction

High grade malignant gliomas are among the most aggressive and deadly tumours. High grade MGs include such tumour types as anaplastic astrocytoma and anaplastic oligodendroglioma and GBM. These MGs vary in life expectancy but even with optimal care survival does not exceed 1.5 years for GBM and 5 years for the less aggressive forms [13]. Because of their refractiveness to conventional treatments including surgery, radiation and chemotherapy and because of their highly sensitive location – the brain – novel experimental therapies are sought, such as immunotherapy, in hopes they offer a survival advantage.

Immunotherapy by exogenous administration of immune cells or immunoregulatory factors, offers a powerful tool which can allegedly destroy tumour cells and spare the healthy tissue. In this paper we model of MG treatment with adoptive alloreactive CTLs as performed in a clinical trial [15, 16] and following the ideas presented in Kronik et al. [14]. To obtain a clearer view of how to apply immunotherapy to MG patients a mathematical understanding of the system behaviour is mandatory. The dynamics of the tumour-immune cell interactions are complex, including cytotoxic function, immune cell cytokine modulation, extra cellular matrix migration, negative and positive feedbacks by paracrine and autocrine factors. As the central nervous system (CNS) is isolated by a selective blood brain barrier (BBB), it provides the tumour an immunologic privilege by excluding many components of the peripheral immune system [10]. Only activated T lymphocytes gain entry to the brain and even then they are short-lived [10]. Cells within the CNS may function as antigen presenting cells of the tumour cell by presenting tumour antigens (Ags) on major histocompatibility complex (MHC) class II molecules thus mediating lymphocytic CD4+ T-helper recognition. Tumour cells enable recognition by means of Ags presentation on MHC class I molecules on their surface. However, immune cells that reach the MG itself often die out without performing their expected task [2, 11, 19, 22]. This is probably due to the immunosuppressive role of the cytokine TGF-β, whose levels are naturally high in the CNS [10]. The cytokine TGF-β suppresses the production of MHC class II [23], as well as the activation and proliferation of CTLs [24]. This down regulation of the immune reaction can be balanced by cytokines, such as interferonγ (IFN-γ), which increase MHC class I and MHC class II molecules expression by tumour cells [18, 21, 25]. Carpentier et al. [6] have shown that IFN-γ (together with TNFα) increases MHC class II expression on the surface of astrocytes, as well as T cell migration across the BBB vascular mobility [10]. Lately Kruse et al. [16] have shown alloreactive CTL infusions to be efficient treatment in MG. We include their suggested therapy in the model.

Over the past thirty years the complex biological dynamics involved in cancer growth and therapeutics have been studied by mathematical modelling, e.g., [1, 3, 7, 20]. In particular, theoretical models of cancer immunology and immunotherapy have been put forward by, e.g., Kuznetsov et al. [17], focusing on the innate CTL response to the growth of an immunogenic
tumour, and Kirschner and Panetta [12], modelling adoptive immunotherapy and predicting its efficacy. Other insightful studies are de Pillis et al. [8] and de Pillis et al. [9]. The first study describes an immunotherapy model using autologous natural killer cells and CD8+ cells. The second model includes the effect of chemotherapy and tumour vaccine. Arciero et al. [4] analyse tumour antigenicity and the endogenous immune response it invokes in a simple model. In the present work we will examine adoptive effector cell treatments in a model which, unlike Arciero et al. [4], considers the involvement of MHC class I and II receptors in the immune reaction. Moreover, recently, Cappuccio et al. [5] have put forward a mathematical model of immunotherapy with interleukin-21 and have retrospectively validated it by experimental results in cancer bearing animals. Brain cancer immunotherapy by intra-tumoural alloreactive CTLs is probably the most promising immunotherapy for MG, due to the impermeability of the BBB. In this work we presented a mathematical model for MG adoptive immunotherapy, which apprises alloreactive CTLs and MHC receptors. The analysis of such a model should allow a prediction of strategic timing and indicate where intervention can lead to tumour control and even cure.

In the following we present the model (Section 2), consider its scaling, boundedness and dissipativity (Section 3). Next, in Section 4 we study untreated steady states. Finally, in Section 5 we study the steady states attainable by treatments.

Section 6 is focused on the simplified version of the model proposed in Section 2. We perform the complete analysis for the simplified system, without and with a constant infusion treatment. Additionally, we estimate a time needed to cure the disease under the assumption of constant infusion of CTLs (Subsection 6.4). Subsection 6.5 is focused on the more realistic protocol with pulse treatment. Finally, we show that the solutions to the model with constant inflow of CTLs underestimate and overestimate solutions to the simplified model with quasi steady approximation for TGF-β. In Section 7 we present summary and discussion of the obtained results.

2 Presentation of the model

In this section we present the model which essentially follows the ideas presented in [14]. However, we propose the more general formulation in which we do not assume the specific forms of functions describing the right-hand side of the system, as in [14]. We describe the interaction between immune system and tumour cells in the case of brain tumour glioblastoma multiforme (GBM), including the possibility of treatment by external infusion of alloreactive CTLs.

We introduce 6 variables describing concentrations of the main components of the analysed processes, namely T(t) reflecting tumour size, C(t) describing the number of CTLs, Fβ and Fγ are concentrations of TGF-β and INF-γ, respectively, and MI and MII represents molecules of MHC class I and II, respectively.

The system of equations studied in this paper has the following form:

\[
\begin{align*}
\dot{T} &= r(T)T - f_T(F_\beta)g_T(MI)h(T)CT, \\
\dot{C} &= f_C(T \cdot MII)g_C(F_\beta) - \mu_C C + S(t), \\
\dot{F_\beta} &= f_\beta(T) - \mu_\beta F_\beta, \\
\dot{F_\gamma} &= f_\gamma(C) - \mu_\gamma F_\gamma, \\
MI &= f_{MI}(F_\gamma) - \mu_{MI} MI, \\
MII &= f_{MII}(F_\beta)g_{MII}(F_\gamma) - \mu_{MII} MII,
\end{align*}
\]  

where:
• \( r(T) \) describes the rate of tumour growth;
• the functions \( f_T(F_\beta) \) and \( g_T(F_{MI}) \) describes the CTL efficiency;
• \( h(T) \) is the tumour damping function;
• \( f_C(T \cdot MII) \) is the CTL production function;
• \( S(t) \) is the treatment function describing the infusion of alloreactive CTLs;
• \( f_\beta(T) \) and \( f_\gamma(C) \) are the production functions of TGF-\( \beta \) and IFN-\( \gamma \), respectively;
• \( f_{MI}(F_\gamma) \) is the MHC class I production function;
• \( g_{MII}(F_\gamma) \) and \( f_{MII}(F_\beta) \) are the MHC class II production and inhibition, respectively.

3 Basic properties

In this section we present the basic properties of the system (1). We study this system with non-negative initial data and the functions \( r, f_T, g_T, h_T, f_C, g_C, S, f_\beta, f_\gamma, f_{MI}, f_{MII} \) and \( g_{MII} \) of class \( C^1 \) and non-negative values. These assumptions guarantee the existence and uniqueness of positive solutions to the system (1). We also need some additional assumptions to avoid blow ups. Therefore, we formulate the first list of assumptions (A1):

1. \( r(T) \) is at most linear, i.e. \( r(T) \leq r, r = \text{const} \) (in the following we study the model with \( r(T) = r(1 - \frac{T}{K}) \));
2. \( f_T(F_\beta) \) and \( g_T(F_{MI}) \) have the properties: \( f_T \) is decreasing and bounded from below, \( g_T \) is increasing and bounded from above;
3. \( h_T \) is decreasing and bounded from below;
4. \( f_C(T \cdot MII) \) is increasing and bounded from above, while the inhibition of CTL production \( g_C(F_\beta) \) is decreasing and bounded from below;
5. \( S(t) \) is non-negative and bounded from above;
6. \( f_\beta(T) \) and \( f_\gamma(C) \) are increasing;
7. \( f_{MI}(F_\gamma) \) is increasing and bounded from above with respect to the concentration of \( \text{INF-}\gamma \);
8. \( g_{MII}(F_\gamma) \) is increasing and bounded from above, while \( f_{MII}(F_\beta) \) is decreasing and bounded from below.

The assumptions (A1) imply that all solutions to Eqs. (1) are non-negative for non-negative initial data and they are defined for every \( t \geq 0 \).

To study the behaviour of the system we need to specify another properties of the functions describing the right-hand side of Eqs. (1). Moreover, to simplify the notation we use the
following new variables: $x = F_\beta$, $y = F_\gamma$, $u = MI$, $v = MII$ and function’s indexes: $f_\beta = f_x$, $f_\gamma = f_y$, $f_{MI} = f_u$, $f_{MII} = f_v$ and $g_{MII} = g_v$. The system (1) takes the following form:

$$
\begin{align*}
\dot{T} &= r(T)T - f_T(x)g_T(u)h_T(T)TC, \\
\dot{C} &= f_C(Tv)g_C(x) - \mu_C C + S, \\
\dot{x} &= f_x(T) - \mu_x x, \\
\dot{y} &= f_y(C) - \mu_y y, \\
\dot{u} &= f_u(y) - \mu_u u, \\
\dot{v} &= f_v(x)g_v(y) - \mu_v v,
\end{align*}
$$

(2)

for which we assume the following biologically based properties (A2):

1. $r(T)$ is decreasing on $[0, K]$, $r(0) = r$ and $r(K) = 0$, where $K$ is the carrying capacity (maximal size) of the tumour. We also assume that for $0 \leq T \leq K$ $r''(T) \geq 0$. This means that the growth rate is decreasing with tumour load, but the rate of decrease is not growing. For the logistic growth $r(T) = r(1 - \frac{T}{K})$ there is $r''(T) = 0$;

2. $f_T(x)$ is decreasing, $f_T(0) = 1$ and $\lim_{x \to \infty} f_T(x) = a_{T,x} > 0$ (TGF-$\beta$ decreases the efficacy of tumour kill by CTLs, up to some limit);

3. $g_T(u)$ is increasing, $g_T(0) = 0$, and $\lim_{u \to \infty} g_T(u) = a_T > 0$ (MHC class I receptors are necessary for CTLs action and increases their efficiency up to some limit);

4. $h_T(T)$ is decreasing, $h_T(0) = 1$ and $\lim_{T \to \infty} h_T(T) = 0$ (large tumour mass hampers CTLs access to the tumour cells and therefore reduces the kill rate);

5. $f_C(Tv)$ is increasing from 0 to $a_{C,v} > 0$, $f_C'(0) > 0$ and $\lim_{T \to \infty} f_C'(Tv) = 0$ (total number of MHC class II receptors on all tumour cells determines the recruitment of CTLs, the rate of CTL entrance is limited and its growth decreases to zero);

6. $g_C(x)$ is decreasing from 1 to $\frac{a_{C,x}}{\mu_{C,x}} > 0$ (TGF-$\beta$ reduces recruitment of CTLs);

7. $f_x(T) = g_x + a_{x,T}T$, $f_y(C) = a_{y,C}C$ with $a_{x,T}, a_{y,C} > 0$ (TGF-$\beta$ and IFN-$\gamma$ are secreted by the tumour cells and CTLs respectively at constant rate, there is base level secretion of TGF-$\beta$);

8. $f_u(0) = g_u > 0$ and $\lim_{y \to \infty} f_u(y) = g_u + a_{u,y}$, $a_{u,y} > 0$ (there is a constant basic production of MHC class II receptors at the cell surface, while IFN-$\gamma$ increases this production up to some level);

9. $f_v(0) = 1$ and $f_v(x)$ is decreasing to 0 (TGF-$\beta$ decreases MHC class II production to 0);

10. $g_v(0) = 0$ and $g_v(y)$ is increasing to $a_{u,y} > 0$, $g'_v > 0$ and $\lim_{y \to \infty} g'_v(y) = 0$ (IFN-$\gamma$ is necessary to induce production of MHC class II receptors and increases it up to some level, with increase declining to zero);

11. $0 \leq S(t) \leq S_0$ and in the analysis presented in Sections 4 – 5 the function $S$ will be mainly assumed constant in time (either 0 or positive).

Under the last assumptions (A2) the solutions for non-negative initial data have the following properties.
I For the first equation it is obvious that $\dot{T} \leq r(T)T$. Therefore, $T \leq \max\{K,T(0)\}$, as in the case of logistic equation. We model the growth of tumour and hence, $T(t) < K$ for every $t \geq 0$.

II The maximal value of the expression $f_C(Tv)g_C(x)$ is equal to $c_{\max} = a_{C,v}$. Therefore, $\dot{C} \leq c_{\max} + S_{\max} - \mu_C C$ which implies $C \leq C_{\max} = \max\{c_{\max} + S_{\max}, C(0)\}$.

III The first variable $T$ is bounded by $K$ and hence, $f_x(T) \leq \bar{f}_x = g_T + a_xTK$. As for the variable $C$ we obtain $x \leq x_{\max} = \max\{\frac{\bar{f}_x}{\mu_x}, x(0)\}$.

IV As above, $y \leq y_{\max} = \max\{\frac{\bar{f}_y}{\mu_y}, y(0)\}$, where $\bar{f}_y = a_{y,C}C_{\max}$.

V Similarly, $u \leq u_{\max} = \max\{\frac{\bar{f}_u}{\mu_u}, u(0)\}$, where $\bar{f}_u = g_u + a_{u,y}$.

VI The maximal value of $f_vg_v$ is equal to $\bar{f}_v = a_{u,y}$ and hence, $v \leq v_{\max} = \max\{\frac{\bar{f}_v}{\mu_v}, v(0)\}$.

All the analysis presented further will be done under the set of assumptions (A2).

**Corollary 1** All coordinates of the solution to the system (2) with non-negative initial data are bounded.

Note that, the above properties are independent on the explicit form of the functions $f_x$ and $f_y$ (the functions are continuous, so they are bounded on the bounded intervals, i.e. for $T \in [0,K]$ and $C \in [0,C_{\max}]$).

**Theorem 1** The system (2) is dissipative in $[0,K] \times (\mathbb{R}^+)^5$ under the assumptions (A2).

**Proof:** Let $W(T,C,x,y,u,v) = T + C + x + y + u + v$. To obtain dissipativity we would like to have $\nabla W \cdot F \leq A - \delta W$, where $F$ denotes the right-hand side of Eqs. (2), $A$ and $\delta$ are positive constants. Note, that $r(T)T$ is zero at $T = 0$ and at $T = K$, thus we can estimate $r(T)T \leq a - bT$ for some positive constants $a$ and $b$, on this interval. In the case of the logistic function $r(T) = r(1 - \frac{T}{K})$ we have $r(T)T \leq Kr - rT$. Hence,

$$
\nabla W \cdot F =
$$
$$
= r(T)T - f_T(x)g_T(u)CTh_T(T) + f_C(Tv)g_C(x) - \mu_C C + S + f_x(T) + 
- \mu_x x + f_y(C) - \mu_y y + f_u(y) - \mu_u u + f_v(x)g_v(y) - \mu_v v 
\leq a - bT + c_{\max} - \mu_C C + S_0 + \bar{f}_x - \mu_x x + \bar{f}_y - \mu_y y + \bar{f}_u - \mu_u u + \bar{f}_v - \mu_v v 
\leq A - \delta W,
$$

where $\delta = \min\{b,\mu_C,\mu_x,\mu_y,\mu_u,\mu_v\}$ and $A = a + c_{\max} + S_0 + \bar{f}_x + \bar{f}_y + \bar{f}_u + \bar{f}_v$. 

**Corollary 2** The system (2) has a compact global attractor in $[0,K] \times (\mathbb{R}^+)^5$.

Note that for the logistic $r(T)$ the linear estimation $r(T)T \leq a - bT$, $a, b > 0$ is valid for every $t \geq 0$ and therefore, the system (2) has a compact global attractor in $(\mathbb{R}^+)^6$. However, due to biological meaning of the parameter $K$ which is the maximal tumour size, we consider only $T \in [0,K]$ (tumour cannot grow beyond its maximal size).
4 Steady states — untreated case

Now, we focus on the existence and stability of steady states for the case without a treatment, that is \( S \equiv 0 \).

From the first equation of Eqs. (2) it is obvious that either \( T = 0 \) at the steady state or

\[
    r(T) = f_T(x)g_T(u)h_T(T)C \quad \text{for} \quad T \neq 0. \tag{3}
\]

In the following subsections we show that the system (2) have two or more steady states depending on the model parameters. Studying its stability we calculate the Jacobi matrix of Eqs. (2):

\[
\begin{pmatrix}
    r'(T)T + r(T) - f_T(x)g_T(u) & -f_T(x)g_T(u) & -f_T(x)g_T(u) & 0 & -f_T(x)g_T(u) & 0 \\
    -f_T(x)g_T(u)C & -h_T(T)T & -h_T(T)CT & 0 & 0 & 0 \\
    (h_T(T) + Th'_T(T)) & 0 & 0 & 0 & 0 & 0 \\
    f'_C(Tv)g_C(x)v & -\mu_C & f_C(Tv)g'_C(x) & 0 & 0 & f'_C(Tv)g_C(x)T \\
    f'_x(T) & 0 & -\mu_x & 0 & 0 & 0 \\
    0 & f'_y(C) & 0 & -\mu_y & 0 & 0 \\
    0 & 0 & 0 & f'_u(y) & -\mu_u & 0 \\
    0 & 0 & f'_v(x)g_v(y) & f_v(x)g'_v(y) & 0 & -\mu_v \\
\end{pmatrix}
\tag{4}
\]

4.1 Semi-trivial tumour-free steady state

Assume that \( T = 0 \). Then

- \( C = 0 \) due to \( f_C(0) = 0 \);
- \( x = \frac{f_x(0)}{\mu_x} = \frac{\mu_x}{\mu_x} > 0 \);
- \( y = 0 \) due to \( f_y(0) = 0 \);
- \( u = \frac{f_u(0)}{\mu_u} = \frac{\mu_u}{\mu_u} > 0 \);
- \( v = 0 \) due to \( g_v(0) = 0 \).

Therefore, there exists the unique semi-trivial tumour-free steady state \((0, 0, \frac{\mu_x}{\mu_x}, 0, \frac{\mu_u}{\mu_u}, 0)\). For the tumour-free steady state we have only one non-zero term in the first row of the Jacobi matrix (4) which is equal to the first eigenvalue of the system. Hence, \( \lambda_1 = r(0) = r > 0 \) and this implies instability of this state.

4.2 Semi-trivial tumour-present steady state

Coming back to Eq. (3) we see that \( T = K \) and \( C = 0 \) satisfy it. From the second equation of Eqs. (2) under the condition \( S \equiv 0 \), there should be \( f_C(Tv)g_C(x) = \mu_C C \) at any steady state. Using the assumptions \( f_y(0) = 0, g_v(0) = 0 \) and \( f_C(0) = 0 \) we see that \( C = y = v = 0 \).
Hence, there is the second semi-trivial tumour-present steady state \((K, 0, x_K, 0, u_K, 0)\), where \(x_K = \frac{f_x(K)}{\mu_x}\) and \(u_K = \frac{f_u}{\mu_u}\).

The Jacobi matrix in this case takes the following form:

\[
\begin{pmatrix}
-r - f_T(x_K) g_T(u_K) h_T(K) K & 0 & 0 & 0 & 0 \\
0 & -\mu_C & 0 & 0 & 0 \\
f'_x(K) & 0 & -\mu_x & 0 & 0 \\
0 & f'_y(0) & 0 & -\mu_y & 0 \\
0 & 0 & f'_u(0) & -\mu_u & 0 \\
0 & 0 & 0 & f_v(x_K) g'_v(0) & -\mu_v
\end{pmatrix}
\]  

(5)

Calculating the characteristic polynomial for the steady state above one gets:

\[
P(\lambda) = (\lambda + r)(\lambda + \mu_x)(\lambda + \mu_u)[(\lambda + \mu_y)(\lambda + \mu_v)(\lambda + \mu_C) - A]
\]

where

\[
A = f'_y(0) f'_v(0) g_C(x_K) f_v(x_K) g'_v(0) K,
\]

(6)

Since \(r, \mu_x\) and \(\mu_u\) are positive, the necessary condition of stability of the tumour-present semi-trivial steady state is negativeness of the roots of the polynomial

\[
Q(\lambda) = (\lambda + \mu_y)(\lambda + \mu_v)(\lambda + \mu_C) - A
\]

From the assumptions (A2), it follows, that \(A\) is non-negative. For \(A = 0\), all three roots of \(Q\) are negative real (since \(\mu_y, \mu_v\) and \(\mu_C\) are positive). Upon increasing \(A\), the maximal root increases towards 0, while two other roots remain negative, or become complex with negative real part (since the sum of the three roots remains unchanged while \(A\) is increasing). Thus, for \(A < \mu_y \mu_v \mu_C\) all three roots have negative real part, while at \(A = \mu_y \mu_v \mu_C\), the maximal root becomes 0 and the steady state looses its stability when \(A > \mu_y \mu_v \mu_C\).

**Corollary 3** If

\[
A < \mu_y \mu_v \mu_C,
\]

(7)

where \(A\) is defined by Eq. (6), then the semi-trivial tumour-present steady state is locally asymptotically stable. The inverse inequality implies instability of this state.

### 4.3 Positive steady state

Let \((T_p, C_p, x_p, y_p, u_p, v_p)\) denote a steady state with \(T_p > 0\). Then

\[
\begin{aligned}
x_p &= \frac{f_x(T_p)}{\mu_x}, & y_p &= \frac{f_y(C_p)}{\mu_y}, & u_p &= \frac{f_u(y_p)}{\mu_u}, & v_p &= \frac{f_v(x_p) g_v(y_p)}{\mu_v}
\end{aligned}
\]

This means that if there exist \(T_p > 0\) and \(C_p > 0\), then the positive steady state is defined. Therefore, we need to solve the system of equations

\[
\begin{aligned}
r(T_p) &= f_T \left( \frac{f_x(T_p)}{\mu_x} \right) g_T \left( \frac{f_u(C_p)}{\mu_u} \right) h_T(T_p) C_p, \\
f_C \left( \frac{f_v \left( \frac{f_x(T_p)}{\mu_x} \right) g_v \left( \frac{f_u(C_p)}{\mu_u} \right) T_p}{\mu_v} \right) g_C \left( \frac{f_x(T_p)}{\mu_x} \right) &= \mu_C C_p.
\end{aligned}
\]

(8)

(9)
Let us consider Eq. (9) and define the auxiliary function

\[
H_T(C) = f_C \left( \frac{f_x(T)}{\mu_x} g_v \left( \frac{f_y(C)}{\mu_y} \right) T \right) g_C \left( \frac{f_x(T)}{\mu_x} \right) - \mu_C C
\]

with the non-negative parameter \( T \geq 0 \).

We see that for every \( T \geq 0 \) there is \( H_T(0) = 0 \). Hence, if \( H_T \) is a strictly monotonic function, then there is no positive steady state. Taking the derivative with respect to \( C \) one gets:

\[
H'_T(C) = f'_C \left( \frac{f_x(T)}{\mu_x} g_v \left( \frac{f_y(C)}{\mu_y} \right) T \right) f_v \left( \frac{f_x(T)}{\mu_x} \right) g_v' \left( \frac{f_y(C)}{\mu_y} \right) f_y(C) g_C \left( \frac{f_x(T)}{\mu_x} \right) - \mu_C.
\]

For a given \( T \), if for all \( C \) there is \( H'_T(C) < 0 \), then no positive solution exists. We recall from the assumptions (A2) the following properties: \( f'_C \) and \( g'_v \) are decreasing to 0 and \( f'_y = \text{const} \). Under these assumptions, \( H'_T(C) \) is decreasing, thus the condition \( H'_T(0) < 0 \) is sufficient to provide \( H'_T(C) < 0 \) for any \( C \). On the other hand, if \( H'_T(0) > 0 \), the above assumptions imply that for \( C \) big enough there is \( H'_T(C) < \epsilon - \mu_C \) with \( \epsilon \) arbitrarily small, thus the equation \( H_T(C) = 0 \) has a positive solution. Moreover, the \( H'_T(C) \) is decreasing (the second derivative has a constant sign), thus there is only one positive \( C \) for which \( H_T(C) = 0 \).

Using the assumptions (A2) we can obtain the following estimation for \( 0 \leq T \leq K \):

\[
H'_T(0) < \frac{f'_C(0)f_v(x_K)Kg_v'(0)f'_y(0)g_C(x_K)}{\mu_v\mu_y} - \mu_C,
\]

where \( x_K = \frac{f_x(K)}{\mu_x} \) is the \( x \)-coordinate of the semi-trivial tumour-present steady state.

**Corollary 4** If \( A < \mu_y\mu_v\mu_C \), that is Ineq. (7) is satisfied, then there is no positive steady state with \( 0 < T < K \).

Corollaries 3 and 4 suggest that for \( A = \mu_y\mu_v\mu_C \) we can expect the switch of stability between the semi-trivial tumour-present and positive steady states.

## 5 Steady states for the system with treatment

In this section we explore steady states and stability of the system (2) for a positive constant \( S \).

### 5.1 Semi-trivial steady state and tumour elimination

For the system (2) with positive \( S \) the steady-state value of \( C \) is non-zero, thus the only semi-trivial case occurs when \( T_{st} = 0 \). Then

\[
C_{st} = \frac{S}{\mu_C}, \quad x_{st} = \frac{f_x(0)}{\mu_x}, \quad y_{st} = \frac{f_y(C_{st})}{\mu_y}, \quad u_{st} = \frac{f_u(y_{st})}{\mu_u}, \quad v_{st} = \frac{f_v(x_{st})g_v(y_{st})}{\mu_v}
\]
and all other coordinates are positive. To examine the stability we note that the Jacobi matrix for Eqs. (2) with positive $S$ remains the same as in the untreated case. For the steady state $(0, C_{st}, x_{st}, y_{st}, u_{st}, v_{st})$ one gets:

$$
\begin{pmatrix}
  r - g_T(u_{st})f_T(x_{st})C_{st} & 0 & 0 & 0 & 0 & 0 \\
  f_C(0)g_C(x_{st})v_{st} & -\mu_C & 0 & 0 & 0 & 0 \\
  f_x(0) & 0 & -\mu_x & 0 & 0 & 0 \\
  0 & f_y(C_{st}) & 0 & -\mu_y & 0 & 0 \\
  0 & 0 & f_y(x_{st})g_y(v_{st}) & f_y(x_{st})g_y(v_{st}) & 0 & -\mu_v \\
  0 & 0 & 0 & 0 & 0 & 0
\end{pmatrix}
$$

(10)

It is easy to see, that the eigenvalues of the matrix (10) are equal to $-\mu_v, -\mu_u, -\mu_y, -\mu_x, -\mu_C$ and $r - g_T(u_{st})f_T(x_{st})C_{st}$, of which the first five are always negative. For the last expression, we observe that $r$ and $f_T(x_{st})$ depend on parameter values only, while $g_T(u_{st})C_{st}$ is increasing (at least linearly) in $S$. Therefore, for $S$ large enough the last eigenvalue is also be negative.

**Corollary 5** Let $S$ be large enough, such that

$$
\frac{S}{\mu_C}g_T \left( \frac{f_u (S/\mu_C)}{\mu_u} \right) > \frac{r}{f_T (f_x(0))}.
$$

Then the semi-trivial steady state $(0, C_{st}, x_{st}, y_{st}, u_{st}, v_{st})$ is locally asymptotically stable.

Corollary 5 gives a necessary condition for the treatment to be efficient in tumour elimination. Further, for large enough value of $S$ one can obtain global stability of this steady state. Indeed, let $S = S^*$, then starting from some moment, $C > C^* = \frac{S^*}{\mu_C}(1 - \epsilon)$ for $\epsilon$ arbitrarily small. Consequently, if we put $y^* = \frac{f_y(C^*)}{\mu_y}(1 - \epsilon_1)$ and $u^* = \frac{f_u(y^*)}{\mu_u}(1 - \epsilon_2)$, for small $\epsilon_1, \epsilon_2$, then, starting from some moment, $u > u^*$ and therefore, $g_T(u)C > g_T(u^*)C^*$. Note also, that $g_T(u^*)C^*$ increases in $S^*$ at least linearly. On the other hand, values of $f_T$ and $h_T$ are bounded from below: $h_T(T) \geq h_T(K)$ and $f_T(x) \geq f_T(x_{max})$. Therefore, if we can supply the system with $S^*$ large enough, such that $g_T(u^*)C^* > \frac{r}{h_T(K)f_T(x_{max})}$, then $\dot{T} \leq -aT$, where $a = g_T(u^*)C^*-\frac{r}{h_T(K)f_T(x_{max})} > 0$, and therefore, the tumour size decays to 0.

**Remark 1** If $\frac{S}{\mu_C}g_T \left( \frac{f_u (S/\mu_C)}{\mu_u} \right) > \frac{r}{f_T (f_x(0))h_T(K)}$, then the tumour size decays exponentially to 0.

Comparing to Corollary 5, the condition assumed in Remark 1 is stronger and requires larger value of injection rate $S$.

### 5.2 Positive steady state

To find a positive steady state $(T_p^t, C_p^t, x_p^t, y_p^t, u_p^t, v_p^t)$ for the system (2) with treatment one needs to solve the system of equations, where the first one is Eq. (8) and the second is the modification of Eq. (9):

$$
\left( f_C \left( \frac{T_p^t f_v (f_x(T_p^t))}{\mu_v} \right) g_C \left( \frac{f_x(T_p^t)}{\mu_x} \right) \right) g_C \left( \frac{f_x(T_p^t)}{\mu_x} \right) + S = \mu_C C_p^t.
$$

(11)
Defining, similarly to the previous section,

\[
\tilde{H}_T(C) = f_C \left( \frac{f_v \left( \frac{f_x(T)}{\mu_x} \right) g_v \left( \frac{f_u(C)}{\mu_y} \right) T}{\mu_v} \right) g_C \left( \frac{f_x(T)}{\mu_x} \right) + S - \mu_C C,
\]

and observing that \( \tilde{H}'_T(C) = H'_T(C) \) while \( \tilde{H}_T(0) = S > 0 \), we conclude, that for each \( T \) there is exactly one \( C \) that solves Eq. (11). The existence of solution to the system (8)-(11) depends on specific properties of the functions involved in the system and parameters.

6 Simplified versions of the system (2)

In this section we consider a simplification of the system (2) to facilitate the analysis and to compare the dynamics of the system without treatment to the system with an external input of CTLs. We introduce the following assumption:

(A3) Dynamics of TGF-β is much faster than those of other system components.

This assumption is based on turnover and secretion rate estimated for TGF-β from experimental data. Therefore, the variable \( x \) is determined as \( x = \frac{f_x(T)}{\mu_x} \). Let define

\[
\bar{f}_T(T) = f_T \left( \frac{f_x(T)}{\mu_x} \right), \quad \bar{g}_C(T) = g_C \left( \frac{f_x(T)}{\mu_x} \right) \quad \text{and} \quad \bar{f}_v(T) = f_v \left( \frac{f_x(T)}{\mu_x} \right).
\]

Then the system (2) takes the following form:

\[
\begin{align*}
\dot{T} & = r(T)T - \bar{f}_T(T)g_T(u)h_T(T)TC, \\
\dot{C} & = f_C(Tv)\bar{g}_C(T) - \mu_C C + S, \\
\dot{y} & = f_y(C) - \mu_y y, \\
\dot{u} & = f_u(y) - \mu_u u, \\
\dot{v} & = \bar{f}_v(T)g_v(y) - \mu_v v.
\end{align*}
\]

All the analysis in this section is performed using the assumption (A3), thus we analyse the system (12).

6.1 Simplification of natural CTL production function

Furthermore, we neglect the influence of MHC class II receptors and TGF-β on natural CTL inflow. Thus we assume:

(A4) The inflow of CTLs is constant.

The constant inflow rate, \( S \), serve us in two different setups. First, in the untreated system we assume that \( S \) is small or even equal to zero, which can be justified for immunosuppressive tumours, e.g. GBM. On the other hand, in treated case we consider a constant CTL infusion for a given amount of time and determine, whether such treatment eliminates the tumour.

According to the assumption (A4), the system (12) becomes:

\[
\begin{align*}
\dot{T} & = r(T)T - \bar{f}_T(T)g_T(u)h_T(T)TC, \\
\dot{C} & = S - \mu_C C, \\
\dot{y} & = f_y(C) - \mu_y y, \\
\dot{u} & = f_u(y) - \mu_u u, \\
\dot{v} & = \bar{f}_v(T)g_v(y) - \mu_v v.
\end{align*}
\]

(13)
Note that the last equation in the system (13) became uncoupled and we can omit it in our analysis, being primarily interested in dynamics of $T$ and $C$. In the following three subsections we study the resultant system of four equations:

$$
\dot{T} = r(T)T - \tilde{f}_T(T)g_T(u)h_T(T)TC,
\dot{C} = S - \mu_C C,
\dot{y} = f_g(C) - \mu_y y,
\dot{u} = f_u(y) - \mu_u u.
$$

\[ \text{(14)} \]

### 6.2 Untreated case

We first study the case when $S = 0$, that is there is no inflow of CTL (highly immunosuppressive tumour). Then, for any initial number of CTLs, $C$ converges to zero exponentially fast, as well as $y$, while $u$ converges exponentially fast to its unique steady state $\frac{L_u}{\mu_u}$. Therefore, the second term on the right-hand side of the first equation of Eqs. (13) is arbitrarily small after enough time, so $T$ grows according to the logistic law to the carrying capacity, $K$.

We conclude that in the untreated case with $S < 0$ the system (13) always converges to the semi-trivial steady-state $(K, 0, \frac{L_u(K)}{\mu_u}, 0, \frac{L_u}{\mu_u})$.

### 6.3 Treated case — constant inflow of CTLs

Now we consider the non-zero inflow, either for small $S$ (constant natural low inflow of CTLs) or for large $S$ (treatment by constant infusion over some time).

To study the asymptotic behaviour of the system (14) we observe that the steady states for the last three equations are: $C^* = \frac{S}{\mu_C}$, $y^* = \frac{L_u(C^*)}{\mu_y}$ and $u^* = \frac{L_u(y^*)}{\mu_u}$, respectively. Therefore, steady states of this system can be found by solving the equation:

$$
r(T^*)T^* - \tilde{f}_T(T^*)g_T(u^*)h_T(T^*)T^*C^* = 0.
$$

\[ \text{(15)} \]

Thus we have $T^*$ given either by $T^* = 0$ or by

$$
r(T^*) - \tilde{f}_T(T^*)g_T(u^*)h_T(T^*)C^* = 0.
$$

\[ \text{(16)} \]

To analyse this equation we denote:

$$
G(S) = g_T(u^*(S))C^*(S), \quad H(T) = \tilde{f}_T(T)h_T(T).
$$

\[ \text{(17)} \]

Eq. (16) becomes:

$$
r(T^*) - G(S)H(T^*) = 0,
$$

\[ \text{(18)} \]

where $G(S)$ is increasing in $S$ at least linearly, while both $r(T)$ and $H(T)$ are decreasing.

First, we note that $r(K) = 0$ and $H(K) = \tilde{f}_T(K)h_T(K) > 0$. Defining the left-hand side of Eq. (18) by $L_S(T) = r(T) - G(S)H(T)$ we can write $L_S(K) < 0$ for every $S > 0$. On the other hand, $L_S(0) = r(0) - G(S)H(0) = r(0) - G(S)\tilde{f}_T(0) = r(0) - G(S)\tilde{f}_T\left(\frac{L_u}{\mu_u}\right)$. Since $G(S)$ is increasing, we can define $S_{min} = G^{-1}\left(\frac{r(0)}{\tilde{f}_T\left(\frac{L_u}{\mu_u}\right)}\right)$. Hence, if $S < S_{min}$, then $L_S(0) > 0$ and $L_S(K) < 0$. Therefore, Eq. (18) has at least one solution $T^* \in (0, K)$, and in general, odd number of solutions $T_1^*, \ldots, T_n^*$. 

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For asymptotic stability analysis we can assume that $C$, $y$ and $u$ are at the steady state, since these variables converge to their steady-state values exponentially and for $t$ large enough the system (14) is arbitrarily well approximated by one equation

$$\dot{T} = r(T)T - H(T)G(S)T = TL_S(T). \quad (19)$$

It is easy to see that for $S < S_{\text{min}}$ the semi-trivial steady state $(0, C^*, y^*, u^*)$ is always unstable, while the first positive steady state $(T^*_1, C^*, y^*, u^*)$ is always locally asymptotically stable and in particular attracts the solutions with smaller initial tumour size.

On the other hand, if $S$ is large enough, that is $S > S_{\text{min}}$, then the semi-trivial steady state is locally stable, since $L_S(0) < 0$. In the general case, however there still could exist positive solutions to Eq. (18), $T^*_1, \ldots, T^*_n$, with $n$ even. Then, arranging them in non-decreasing order, one can see that the state with the tumour level $T^*_i$ is locally stable for even $i$ and unstable for odd $i$ (counting the multiple solutions). Thus, in the case $n = 0$, the only steady state $(0, C^*, y^*, u^*)$ is globally asymptotically stable, while if additional steady states exist, the semi-trivial tumour-free steady state $(T = 0)$ is only locally stable and for large enough initial tumour size the value $T(t)$ converges to some of $T_i$.

Now consider some $S = S_{\text{min}} + \epsilon$, with $\epsilon > 0$, such that the semi-trivial steady state is locally stable. If there are no other steady states than $T = 0$, then our choice of $S$ is sufficient to eliminate the tumour, while reducing it by more than $\epsilon$ renders the semi-trivial steady state unstable. Thus, in this case, $S > S_{\text{min}}$ is the sufficient and almost necessary condition for tumour elimination. If on the other hand, there are additional steady states with different values $T^*_1, \ldots, T^*_n$, we can eliminate them by increasing $S$. More specifically, let $\Delta$ be the maximal value of

$$\frac{r(T)}{G(S_{\text{min}})H(T)} = \frac{r(T)H(0)}{r(0)H(T)}$$

for $0 \leq T \leq K$ (this function is bounded between 0 and $\frac{r(0)}{G(S_{\text{min}})H(K)}$ on $[0, K]$). Choose $S_{\text{thr}}$ such that $G(S_{\text{thr}}) = G(S_{\text{min}})\Delta$ (this is always possible, since $G(S)$ is increasing at least linearly in $S$ and continuous). Then for $S > S_{\text{thr}}$ we have $L_S(T) < 0$ for all $0 \leq T \leq K$ and $(0, C^*, y^*, u^*)$ is now the globally stable steady state.

**Corollary 6** For the system (14), there is always the semi-trivial steady state $(0, C^*, y^*, u^*)$. The asymptotic behaviour of the system is described as follows:

- if $S < S_{\text{min}}$, the semi-trivial steady state is unstable and there exist an odd number of positive steady states $(T^*_i, C^*, y^*, u^*)$, $i = 1, \ldots, n$. For odd $i$ these states are locally asymptotically stable, while for even $i$ they are unstable.

- if $S_{\text{min}} < S < S_{\text{thr}}$, the semi-trivial steady state is locally stable and there exist an even number of positive steady states $(T^*_i, C^*, y^*, u^*)$, $i = 1, \ldots, n$, of which the states with even $i$ are locally stable. In particular, the solutions with large enough initial tumour size are attracted by a positive steady state.

- if $S > S_{\text{thr}}$, the semi-trivial steady state is unique, and it is globally stable. In particular tumour of any size is eventually eliminated.

### 6.4 Estimation of the treatment time

In this subsection we give guidelines to estimation of time to cure under the constant infusion regimen for the system (14). For these estimations, we assume initial data for the worst case...
subsection, the notation $H(T) = \tilde{f}_T(T)h_T(T) = f_T \left( \frac{L(T)}{\mu_u} \right) h_T(T)$. Hence, the first equation of Eqs. (14) has the following form:

$$\dot{T} = T \left( r(T) - H(T)g_T(u)C \right).$$

(20)

Solving the equation for $C$ one gets $C(t) = C^*(1 - e^{-\mu_C t})$. We want to achieve values of $C$ close to $C^* = \frac{S}{\mu_C}$. More precisely, for arbitrary $\varepsilon_1 > 0$ there is the threshold time $t_1 = \frac{1}{\mu_C} \ln \frac{1}{\varepsilon_1}$, such that $C(t) > C^*(1 - \varepsilon_1)$ for $t > t_1$.

The function $f_T$ is increasing, which yields that $\dot{y} > f_y(C^*(1 - \varepsilon_1) - \mu_y y$ for $t > t_1$. As above, we see that for arbitrary $\varepsilon_2 > 0$, there exists $t_2 = \frac{1}{\mu_y} \ln \frac{1}{\varepsilon_2}$, such that $y(t) > \frac{f_y(C^*(1 - \varepsilon_1))}{\mu_y} \left(1 - \varepsilon_2\right)$ for $t > t_1 + t_2$. Similarly, for a given $\varepsilon_3 > 0$ there exists $t_3$ such that $u(t) > \frac{f_u(f_y(C^*(1 - \varepsilon_1)))}{\mu_u} \left(1 - \varepsilon_3\right)$ for $t > t_1 + t_2 + t_3$.

In particular, we conclude that for arbitrary $\varepsilon_1, \varepsilon_2$, there exist $t_0$ such that $C(t) > C^*(1 - \varepsilon_1)$ and $u(t) > u^*(1 - \varepsilon_2) = \frac{f_y(f_y(C^*(1 - \varepsilon_1)))}{\mu_y} (1 - \varepsilon_2)$ (in the last we use continuity of the functions $f_y$ and $f_u$) for $t > t_0$.

Finally, we see that given an arbitrary $\varepsilon^* > 0$, we can find $t^*$, such that $g_T(u^*)C > g_T(u^*)C^*(1 - \varepsilon^*)$ (using continuity of $g_T$) for $t > t^*$. The assumption that the treatment is curative allows us to use the inequality $S > S_{thr} + \delta_1$ for some $\delta_1 > 0$, which implies that there is some $\delta_2 > 0$, such that $r(T) - H(T)g_T(u^*)C^* < -\delta_2$ for all $T$. Therefore, there exist $\varepsilon^*, \delta_3 > 0$ such that

$$r(T) - H(T)g_T(u^*)C^*(1 - \varepsilon^*) < -\delta_3.$$ 

From Eq. (20) it follows that for $t > t^*$ we have $T < T(t^*)e^{-\delta_3(t - t^*)}$ and thus, for a given final value $T_f$ of $T$ we can find $t_f = \frac{1}{\delta_3} \ln \frac{T_f(t^*)}{T_f}$, such that for $t > t^* + t_f$ there is $T(t) < T_f$. If $T_f$ is the size under which the tumour can be considered harmless, this gives the estimation of time to cure under constant inflow of CTLs. The value of $t_f$ can be minimized by proper choice of $\varepsilon^*$.

The estimation above is very general and can be specified given the functions describing the right-hand side of the system (14). Let us assume that $H(T)$ is convex (as in the case of specific functions proposed in [14]). Similarly to the previous subsection we define the auxiliary function

$$L_S(T) = r(T) - g_T(u^*(1 - \varepsilon_3))C^*(1 - \varepsilon_1)H(T) = r(T) - g^*_T H(T).$$

If $H(t)$ is convex, then the function $L_S(T)$ is concave. We would like to bound $L_S(T)$ by a linear function. There are several possibilities of making this linear estimation. We use the following procedure. In the case of concave $L_S(T)$ its graph lies below the tangent to this curve. Hence, taking the tangent at $T = K$ we get

$$L_S(T) \leq \alpha T - \beta, \quad \alpha = L'_S(K) = r'(K) - g^*_T H'(K) \quad \text{and} \quad \beta = KL'_S(K) - L_S(K)$$

with $\alpha > 0, \beta > 0$ and $\alpha T - \beta < 0$ for $T \in [0, K]$.

Therefore, $\dot{T} \leq T(\alpha T - \beta)$ and calculating integrals from $t_0 = 0$ to $t$ one gets

$$\ln \left( \frac{T}{\beta - \alpha T} \frac{\beta - \alpha T_0}{T_0} \right) \leq -\beta t.$$

(21)
The left-hand side of Ineq. (21) is increasing with respect to $T$. Hence, to get the estimation $T < \varepsilon$ it is sufficient to have
\[
\ln \left( \frac{\varepsilon}{\beta - \alpha} \frac{\beta - \alpha T_0}{T_0} \right) \leq -\beta t.
\]
Finally,
\[
t > \frac{1}{\beta} \ln \frac{T_0(\beta - \alpha \varepsilon)}{\varepsilon(\beta - \alpha T_0)} + t_1 + t_2 + t_3, \text{ with } \varepsilon < 1
\]
and
\[
t_4 = \min \left\{ t(\varepsilon, \varepsilon_1, \varepsilon_2, \varepsilon_3) : \varepsilon \in (0, 1), \varepsilon_1 > 0, \varepsilon_2 > 0, \varepsilon_3 > 1 - \frac{f_0(y_1^*)}{f_0(y^*)} \right\},
\]
where $y_1^* = y_1^*(\varepsilon_2)$, $\alpha = \alpha(\varepsilon_1, \varepsilon_2, \varepsilon_3)$ and $\beta = \beta(\varepsilon_1, \varepsilon_2, \varepsilon_3)$.

6.5 Non-constant treatment

In the second equation of the system (12) we have assumed constant influx of CTLs as a mathematical approximation of a real treatment. Assuming more realistic protocol of treatment we give a certain amount $S$ of CTLs every time $t_n = t_0 + n\Delta t$, where $t_0$ is a treatment starting time and $\Delta t = \text{const}$ in the simplest case. We assume that natural inflow is small ($\sim \varepsilon$) such that the immune system cannot fight tumour without a treatment. In such a case, for every initial time $\hat{t}$ and initial amount of CTLs $\hat{C}$ one gets the following solution:
\[
C(t) = \left( \hat{C} - \frac{\varepsilon}{\mu_C} \right) e^{-\mu_C(t-\hat{t})} + \frac{\varepsilon}{\mu_C}. \quad (22)
\]

For $t < t_0$ there is $C(t) = 0$. At $\hat{t} = t_0$ we have the first injection of CTLs equal to $S$ and hence $C(t) = \left( S - \frac{\varepsilon}{\mu_C} \right) e^{-\mu_C t} + \frac{\varepsilon}{\mu_C}$ until $0 < t < t_1 = \Delta t$ (where $t_0 = 0$ for simplicity). As $t$ approaches $t_1$ from the left hand-side, the amount of CTLs approaches $C_1 = C(t_1) = \left( S - \frac{\varepsilon}{\mu_C} \right) e^{-\mu_C \Delta t} + \frac{\varepsilon}{\mu_C}$.

The second injection of CTLs at $t = t_1$ leads to $C(t) = \left( C(t_1) + S - \frac{\varepsilon}{\mu_C} \right) e^{-\mu_C(t-t_1)} + \frac{\varepsilon}{\mu_C}$ until $t_1 < t < t_2$ and so on.

Therefore, for $t = t_n$ we obtain the recurrent formula
\[
C_n = \left( C_{n-1} + S - \frac{\varepsilon}{\mu_C} \right) e^{-\mu_C \Delta t} + \frac{\varepsilon}{\mu_C}
\]
with $C_0 = 0$. If there exists the limit $\lim_{n \to \infty} C_n = C_{\text{lim}}$, then
\[
C_{\text{lim}} = \left( C_{\text{lim}} + S - \frac{\varepsilon}{\mu_C} \right) e^{-\mu_C \Delta t} + \frac{\varepsilon}{\mu_C}
\]
and hence,
\[
C_{\text{lim}} = \frac{\varepsilon}{\mu_C} + \frac{S}{e^{\mu_C \Delta t} - 1}.
\]

From the formula (22) it is easily seen that $C(t)$ is decreasing under the assumption $\hat{C} > \frac{\varepsilon}{\mu_C}$ which is the case under our consideration. Similarly, the sequence $C_n$ is decreasing and this also means that $C_n \to C_{\text{lim}}$ from above.
We want to keep the amount of CTLs under some given threshold $C_{thr} > \frac{\varepsilon}{\mu_C}$. Hence, it is enough that $C_{lim} > C_{thr}$, which leads to the following inequality:

$$\frac{S}{\varepsilon \mu_C \Delta t - 1} > C_{thr} - \frac{\varepsilon}{\mu_C}. \quad (23)$$

Condition (23) links the parameters $\varepsilon$ and $\mu_C$ of the system with the treatment parameters $S$ and $\Delta t$:

1. If a dose $S$ of CTLs cannot exceed some threshold $C_{max}$, then the time between successive injections should be shortened according to (23).

2. If $\Delta t$ cannot be shorter than some given interval $\Delta t_{min}$, then the dose $S$ should increase according to (23).

### 6.6 Relaxing Assumption (A4)

Here we show that the last results can be useful even when the assumption (A4) is unjustified, that is a natural production of CTLs cannot be neglected. Specifically, we show that in the untreated case the system (13) with positive small $S$ underestimates the number of tumour cells, while in the treated case the system (13) with the same value of $S$ as in (12) overestimates this number. Consider the following two systems in variables $(T_j, C_j, y_j, u_j, v_j)$:

$$\begin{align*}
\dot{T}_j &= r(T_j)T_j - f_T(T_j)g_T(u_j)h_T(T_j)T_jC_j, \\
\dot{C}_j &= g_j(v_j, T_j) - \mu_C C_j, \\
\dot{y}_j &= f_y(C_j) - \mu_y y_j, \quad \text{for } j = 1, 2, \\
\dot{u}_j &= f_u(y_j) - \mu_u u_j, \\
\dot{v}_j &= f_v(T_j)g_v(y_j) - \mu_v v_j.
\end{align*} \quad (24)$$

The systems (24)(1)-(2) differs from Eqs. (12) only in one term, that is the inflow of CTLs $g_j(v_j, T_j)$ which can be non-constant.

**Lemma 1** Assume that for the systems (24)(1) and (24)(2), for every $v_1, v_2, T_1, T_2$ non-negative, there is $g_1(v_1, T_1) \geq g_2(v_2, T_2)$.

If $(T_1(0), C_1(0), y_1(0), u_1(0), v_1(0)) = (T_2(0), C_2(0), y_2(0), u_2(0), v_2(0))$, then $C_1 \geq C_2$ and $T_1 \leq T_2$ for every $t$.

**Proof:** Consider the following system in variables $T_d = T_1 - T_2, C_d = C_1 - C_2, y_d = y_1 - y_2, u_d = u_1 - u_2$ and $v_d = v_1 - v_2$:

$$\begin{align*}
\dot{T}_d &= r(T_1)T_1 - f_T(T_1)g_T(u_1)h_T(T_1)T_1C_1 - r(T_2)T_2 + f_T(T_2)g_T(u_2)h_T(T_2)T_2C_2, \\
\dot{C}_d &= g_1(v_1, T_1) - g_2(v_2, T_2) - \mu_C C_d, \\
\dot{y}_d &= f_y(C_1) - f_y(C_2) - \mu_y y_d, \\
\dot{u}_d &= f_u(y_1) - f_u(y_2) - \mu_u u_d, \\
\dot{v}_d &= f_v(T_1)g_v(y_1) - f_v(T_2)g_v(y_2) - \mu_v v_d. \quad (25)
\end{align*}$$

The system (25) is obtained by subtracting (24)(2) from (24)(1) and has initial zero values. By the assumptions of this lemma, the second equation yields that $C_d \geq 0$ for $t \geq 0$ (since $g_1(v_1, T_1) - g_2(v_2, T_2)$ is always non-negative).
Therefore, \( C_1 \geq C_2 \) and, since \( f_y \) is increasing in \( C \), in the same way we obtain \( y_d \geq 0 \). Further, \( f_u \) is also increasing, thus \( u_d \geq 0 \).

For the last part, we rewrite the first equation of Eqs. (25) in the following form:

\[
\begin{align*}
\dot{T}_d &= (r(T_1) - r(T_2))T_1 + r(T_2)T_d - \bar{f}_T(T_1)h_T(T_1)(g_T(u_1)C_1 - g_T(u_2)C_2) - \\
&- g_T(u_2)C_2(f_T(T_1)h_T(T_1)T_1 - \bar{f}_T(T_2)h_T(T_2)T_2) = \\
&- f_T(T_1)h_T(T_1)T_1 [g_T(u_1)C_1 - g_T(u_2)C_2] - \\
&+ T_0 \left[ r(T_2) + \frac{r(T_1) - r(T_2)}{T_d} \right] T_1 - g_T(u_2)C_2 \left( \bar{f}_T(T_1)h_T(T_1) + \\
&+ T_2 \left( \frac{h_T(T_1) - h_T(T_2)}{T_d} \right) \right].
\end{align*}
\]

Notice, that there exist \( T_s, T_{**}, T_{***} \in (T_1, T_2) \) such that

\[
\begin{align*}
\frac{r(T_1) - r(T_2)}{T_d}T_1 &= r'(T_s)T_1, \\
\frac{h_T(T_1) - h_T(T_2)}{T_d} &= h'_T(T_{**}), \\
\frac{\bar{f}_T(T_1) - \bar{f}_T(T_2)}{T_d} &= \bar{f}'_T(T_{***}).
\end{align*}
\]

This allows to rewrite the first equation of Eqs. (25) in the following form:

\[
\dot{T}_d = - \bar{f}_T(T_1)h_T(T_1)T_1 [g_T(u_1)C_1 - g_T(u_2)C_2] + \\
+ T_0 \left[ r(T_2) + r'(T_s)T_1 - g_T(u_2)C_2 \left( \bar{f}_T(T_1)h_T(T_1) + \\
&+ T_2 \left( \frac{h_T(T_1) - h_T(T_2)}{T_d} \right) \right) \right].
\]

To estimate the last expression notice, that the first term of it is non-positive, because \( g_T \) is increasing. In the second term, \( T_d \) is multiplied by the expression which is bounded, due to the assumptions (A2) and boundedness of solutions. Thus, the second term is bounded from above and below by linear functions of \( T_d \). In particular, the trajectory \( T_d(t) \), starting at \( T_d(0) = 0 \) has always non-positive derivative at the point \( T_d = 0 \), and thus never becomes positive. This yields \( T_d \leq 0 \), as claimed.

Note, that if in this lemma, we assume \( g_1(v_1, T_1) < g_2(v_2, T_2) \), the same proof gives \( C_1 > C_2 \) and \( T_1 < T_2 \).

7 Discussion

In this paper we have proposed and analysed the model of tumour-immune system interactions for GBM brain tumour. The model is based on the ideas presented in Kronik et al. [14] however, we do not assume the specific the specific form of functions describing considered processes. We have started our mathematical analysis by studying the basic properties of the 6 ordinary differential equations system described by Eqs. (2). After defining the necessary characteristics of the system (the assumptions (A2)) we have obtained the boundedness of solutions. Boundedness is important as it shows that the mathematical system has a normal biological behaviour, e.g. that cytokine levels or cell numbers do not drop below 0 and that they do not explode. Moreover, dissipativity in Theorem 1 implies the existence of a global attractor and for some parameter values suggests a global stability of one of the steady states.
Typically, we expect the existence at least one stable steady state. Biologically this means that if a solution starts in a certain neighbourhood around that stable solution, we achieve a stable solution after some time. Therefore, if we achieve a steady state describing either eradication of the tumour or some level of coexistence, the system always returns to that point given a perturbation. We have to ensure, however, that this bounded neighbourhood makes biological sense in the clinical world (cell numbers, cytokine levels, etc.).

Next, we have set to find those steady states in the case with no treatment (Section 4). Are any steady states stable without medical intervention? We have started the analysis with the semi-trivial tumour-free steady state. The state with \( T = 0 \) and \( C = 0 \) is unstable due to its eigenvalue being real and positive. The clinical meaning of an unstable steady state is that in any case when tumour arises in the brain it will inevitably grow and drive the system to explosion, that is a patient’s death. The complementary semi-trivial steady state is for \( T = K \) and \( C = 0 \). This state is stable only when the condition in Corollary 3 is satisfied, namely, that the degradation constants of the pro-inflammatory components of the system (CTLs, IFN-\( \gamma \), MHCII) combined in the coefficient \( A \) are large enough to ensure their removal from the tumour environment. The clinical biological interpretation of this Corollary is that within the range of biological parameters for which \( A < \mu_y\mu_v\mu_C \) the state \( T = K \), that is a patient’s death is an attractor, a state to which the system converges.

We have continued our search for positive steady states without treatment. We have found that the existence of a steady state with \( 0 < T < K \) and \( C > 0 \) requires the fulfilment of the condition \( A > \mu_y\mu_v\mu_C \) for which the semi-trivial tumour-present steady state is unstable. This inequality implies that if the degradation rate of CTLs or IFN-\( \gamma \) or MHCII could be decreased, then such a positive steady state could be maintained.

Next we have turned out to steady states for the system with treatment (Section 5). We have discussed the stability of steady states in which \( 0 < T < K \) when we add the treatment element \( S > 0 \) in the system (2). We have shown that for the semi-trivial steady state to be stable, a large enough \( S \) is required. Biologically this means that to bring about a cure a constant infusion \( S \) of CTLs must be maintained. In our previous work [14] (YK and NK) we have shown simulations of interspersed infusions of aCTLs, however one may simulate such infusions that are bounded by a low constant level of CTLs. The clinical meaning of which is post operative. Once the tumour is reduced to a size within the stable neighbourhood around the \( T = 0 \) steady state, a constant infusion treatment \( S \) will ensure its stable decline towards zero. Another clinical implication is that after the apparent eradication of the tumour by interspersed infusions which may reduce the tumour to very low level we must consider leaving a constant level of \( S \) of aCTLs to ensure the tumour demise.

Looking for positive steady states for the system (2) with treatment we have shown that for each \( T > 0 \) there is exactly one \( C > 0 \) that satisfies the conditions for the existence of positive steady state. The number of positive steady states strongly depends on the specific form of the right-hand side of Eqs. (2)

In Section 6 we have made two assumptions that simplify the mathematical analysis. First, we have assumed that the recruitment function, the patient’s natural MHCII mediated immune reaction, is insufficient to combat the tumour relatively to the large number of aCTLs that we introduce to the system and is negligible. The second critical assumption that we have made is that the turnover rate of TGF-\( \beta \) is much faster (minutes) relatively to the other variable (many hours or days). It is easy to see that such a simplified system in the absence of treatment reaches the semi-trivial steady state with \( T = K \) and \( C = 0 \) and the subsequent patient’s death.

Next, we have turned to study the simplified system with treatment \( S > 0 \). The simpli-
fied system has at least one semi-trivial \((T = 0)\) steady state. The stability of this state is determined by the amount of \(S\). In general the larger \(S\) is the more global is the stability. For \(S < S_{\text{min}}\) the semi-trivial steady state is unstable, that is the solution describing tumour eradication is unstable, such that getting the tumour to be extremely small will not drive the system to cure and the tumour is bound to resurge. However, there can exist other stable steady states with \(T > 0\) depending on other conditions. If \(S_{\text{thr}} > S > S_{\text{min}}\) local asymptotic stability for the cure possibility is secure and even large tumours can be stably held at steady state. Finally, when \(S > S_{\text{thr}}\), then \(T = 0\) steady state is a unique solution and is globally stable meaning that a cure is secured. Biologically this means that a high dose constant infusion of aCTLs given for a certain period of time can bring about a cure for GBM. Nevertheless, an important caveat is maximal applicable CTL dose with causing adverse effects.

If we relax assumption (A4) meaning that the natural recruitment of endogenous CTLs is significant but small, then the untreated case will always underestimate the tumour size. Biologically this means that using the simplified system may lead us to error on the cautious side, and since using more aCTLs increases the stability of the cure solution, unless we reach toxic effect this is a tolerable error.

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**References**


